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In Vitro Activities of U-100592 and U-100766, Novel Oxazolidinone Antibacterial Agents

GARY E. ZURENKO,[1]* BETTY H. YAGI,[1] RONDA D. SCHAADT,[1] JOHN W. ALLISON,[1] JAMES O. KILBURN,[2] SUZANNE E. GLICKMAN,[2] DOUGLAS K. HUTCHINSON,[1] MICHAEL R. BARBACHYN,[1] AND STEVEN J. BRICKNER[1]

Pharmacia & Upjohn, Inc., Kalamazoo, Michigan 49001,[1] and Centers for Disease Control and Prevention, Atlanta, Georgia 30333[2]

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Oxazolidinones make up a relatively new class of antimicrobial agents which possess a unique mechanism of bacterial protein synthesis inhibition. U-100592 {(*S*)-*N*-[[3-[3-fluoro-4-[4-(hydroxyacetyl)-1-piperazinyl]-phenyl]-2-oxo-5-oxazolidinyl]methyl]-acetamide} and U-100766 {(*S*)-*N*-[[3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-acetamide} are novel oxazolidinone analogs from a directed chemical modification program. MICs were determined for a variety of bacterial clinical isolates; the respective MICs of U-100592 and U-100766 at which 90% of isolates are inhibited were as follows: methicillin-susceptible *Staphylococcus aureus*, 4 and 4 μg/ml; methicillin-resistant *S. aureus*, 4 and 4 μg/ml; methicillin-susceptible *Staphylococcus epidermidis*, 2 and 2 μg/ml; methicillin-resistant *S. epidermidis*, 1 and 2 μg/ml; *Enterococcus faecalis*, 2 and 4 μg/ml; *Enterococcus faecium*, 2 and 4 μg/ml; *Streptococcus pyogenes*, 1 and 2 μg/ml; *Streptococcus pneumoniae*, 0.50 and 1 μg/ml; *Corynebacterium* spp., 0.50 and 0.50 μg/ml; *Moraxella catarrhalis*, 4 and 4 μg/ml; *Listeria monocytogenes*, 8 and 2 μg/ml; and *Bacteroides fragilis*, 16 and 4 μg/ml. Most strains of *Mycobacterium tuberculosis* and the gram-positive anaerobes were inhibited in the range of 0.50 to 2 μg/ml. Enterococcal strains resistant to vancomycin (VanA, VanB, and VanC resistance phenotypes), pneumococcal strains resistant to penicillin, and *M. tuberculosis* strains resistant to common antitubercular agents (isoniazid, streptomycin, rifampin, ethionamide, and ethambutol) were not cross-resistant to the oxazolidinones. The presence of 10, 20, and 40% pooled human serum did not affect the antibacterial activities of the oxazolidinones. Time-kill studies demonstrated a bacteriostatic effect of the analogs against staphylococci and enterococci but a bactericidal effect against streptococci. The spontaneous mutation frequencies of *S. aureus* ATCC 29213 were $<3.8 \times 10^{-10}$ and $<8 \times 10^{-11}$ for U-100592 and U-100766, respectively. Serial transfer of three staphylococcal and two enterococcal strains on drug gradient plates produced no evidence of rapid resistance development. Thus, these new oxazolidinone analogs demonstrated in vitro antibacterial activities against a variety of clinically important human pathogens.

The (*S*)-3-aryl-5-acetamidomethyl-2-oxazolidinone antibacterial agents discovered by researchers at E. I. du Pont de Nemours & Co., Inc. were first reported in 1987 (19). An initial screening-derived lead was S-6123 (10), a 5-hydroxymethyl-2-oxazolidinone. While S-6123 exhibited only weak in vitro antibacterial activity, structure-activity optimization led to the more highly active agents DuP-105 {(*S*)-[3-(4-(methylsulfinyl)phenyl)-2-oxo-5-oxazolidinyl)methyl]-acetamide} and DuP-721 {(*S*)-[3-(4-acetylphenyl)-2-oxo-5-oxazolidinyl)methyl]-acetamide} (20). These compounds represented a new class of antibacterial agents which was chemically unrelated to any commercially available agent. The compounds had a number of intriguing attributes, including (i) a unique mechanism of action which involved inhibition of protein synthesis at a very early stage and a lack of cross-resistance with existing antimicrobial agents (9–12); (ii) a spectrum of activity that included a number of important bacterial species (1, 2, 5, 9, 10, 15, 18, 20); (iii) oral and parenteral bioavailability (22) and activity in animal models of human infection (19); (iv) the inability to develop resistant mutants by using various in vitro methodologies (5, 9, 20); and (v) the fact that they were a totally synthetic class with seemingly sufficient structural latitude to allow for structure-activity and structure-toxicity relationship optimization. However, in spite of their desirable characteristics, no antibacterial oxazolidinone has yet been advanced for the treatment of infectious disease in humans.

The potential of this new antibacterial drug class stimulated an exploratory chemical analog program in our laboratories. Two oxazolidinone analogs, U-100592 and U-100766, emerged as superior compounds with the best overall combination of positive attributes. U-100592 and U-100766 are novel 3-(3-fluorophenyl)-2-oxazolidinone antibacterial agents substituted with a 4-(hydroxyacetyl) piperazin-1-yl and a morpholin-1-yl group, respectively (Fig. 1). These oxazolidinone analogs are readily prepared via novel and practical asymmetric syntheses involving, as their key step, the reaction of *N*-lithiocarbamates of appropriate anilines with (*R*)-glycidyl butyrate (4). The resultant (*R*)-5-(hydroxymethyl) oxazolidinones are readily elaborated to U-100592 and U-100766 in excellent yields and in high enantiomeric purities (3). In this report we describe the in vitro antibacterial activities of U-100592 and U-100766 and compare them with those of vancomycin and other reference antibiotics.

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* Corresponding author. Mailing address: Cancer and Infectious Diseases Research, Pharmacia & Upjohn, Inc., 7000 Portage Rd., Kalamazoo, MI 49001-0199. Phone: (616) 385-7049. Fax: (616) 385-6492.

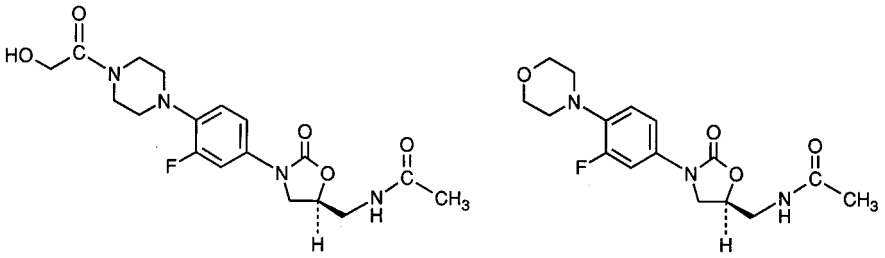

FIG. 1. Chemical structures of U-100592 and U-100766.

MATERIALS AND METHODS

Bacterial strains. The cultures used in the present study were banked bacterial clinical isolates from human infections. The isolates were maintained frozen in the vapor phase of a liquid nitrogen freezer. Recent isolates of vancomycin-resistant enterococci were provided by the following laboratories: The University of Iowa Hospitals and Clinics, Iowa City (Ronald Jones); Hunter Holmes McGuire Medical Center, Richmond, Va. (Philip Coudron); University of Minnesota, Minneapolis (Patricia Ferreri); and Hartford Hospital, Hartford, Conn. (Brian Cooper). Recent isolates of penicillin-resistant pneumococci were provided by the Baylor College of Medicine, Houston, Tex. (Edward Mason, Jr.), and the Hershey Medical Center, Hershey, Pa. (Peter Appelbaum). The reference quality control strains *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Bacteroides fragilis* ATCC 25285 were originally acquired from the American Type Culture Collection (Rockville, Md.).

Antimicrobial agents. U-100592, U-100766, novobiocin sodium, and clindamycin hydrochloride were prepared in our laboratories. Vancomycin, penicillin G, ampicillin sodium, ciprofloxacin hydrochloride, and rifampin were obtained from Sigma Chemical Co. (St. Louis, Mo.).

MIC determinations. (i) Agar dilution method for aerobic bacteria. The MICs for some aerobic bacteria were determined by a standard agar dilution method (16). The media used included Mueller-Hinton agar (MHA; Difco Laboratories, Detroit, Mich.) for nonfastidious organisms, MHA supplemented with 5% defibrinated sheep blood for *Corynebacterium* spp., and proteose no. 3 agar (Difco) supplemented with 1% hemoglobin (Difco) and 1% IsoVitaleX (Becton Dickinson, Inc., Cockeysville, Md.) for *Haemophilus influenzae* and *Moraxella catarrhalis*. These organisms were incubated aerobically at 35°C for 24 h; the corynebacteria, however, were incubated for 42 h.

(ii) Broth microdilution method for aerobic bacteria. The MICs of U-100592, U-100766, vancomycin, and ampicillin for multiple clinical isolates of gram-positive aerobic bacteria were determined by a broth microdilution method (16). Mueller-Hinton broth (MHB; Difco) adjusted with divalent cations to a final concentration of 20 mg of Ca^{2+} per liter and 10 mg of Mg^{2+} per liter (CSMHB) was the test medium for most of the isolates; CSMHB plus 5% lysed horse blood was used to test streptococci and *Listeria monocytogenes.*

The effect of pooled human serum on the activities of U-100592, U-100766, and the control drugs vancomycin, novobiocin, and clindamycin was also determined by the broth microdilution method. Normal pooled human serum was obtained from Clinical Research Laboratories, Pharmacia & Upjohn, Inc., and was stored at −20°C. The MICs were determined in 0, 20, and 40% pooled human serum. The presence of the serum produced MIC endpoints which were difficult to read visually; therefore, after incubation, the reagent alamarBlue (Alamar Biosciences, Sacramento, Calif.) was added at a final concentration of 10% to clarify endpoints. alamarBlue is an oxidation-reduction indicator that detects metabolic reduction of the growth medium. After the addition of the indicator, the plates were shaken on a microplate shaker (Dynatech Laboratories, Chantilly, Va.) for approximately 30 s and were then reincubated at 35°C for 2 h. Visual readings of each of the plates were performed; any change from the blue color of the indicator was considered to be positive culture growth.

(iii) Agar dilution method for anaerobic bacteria. MICs were determined by a reference agar dilution method (17) with Wilkins-Chalgren agar (Difco). The plates were incubated in an anaerobic glove box (Forma Scientific, Inc., Marietta, Ohio) containing an atmosphere of 85% nitrogen, 10% hydrogen, and 5% carbon dioxide for approximately 42 h.

(iv) Agar dilution method for *M. tuberculosis.* The oxazolidinones were incorporated into duplicate plates of 7H10 agar medium supplemented with Middlebrook oleic acid-albumin-dextrose (OADC) enrichment (Difco) at concentrations of 2.0, 0.50, 0.125, and 0.03 μg/ml. Test organisms were grown in 7H9 medium (Difco) containing 0.05% Tween 80. After 7 days of incubation at 37°C, the broths were adjusted to the turbidity of a 1.0 McFarland standard; the organisms were then diluted 10-fold in sterile water containing 0.10% Tween 80. The resulting bacterial suspensions were spotted onto the drug-supplemented 7H10 plates. After a 21-day cultivation at 37°C, the MIC was recorded as the lowest concentration of drug that completely inhibited the growth of the organism. Test isolates included five clinical isolates that were generally susceptible to common antitubercular agents and five strains that were resistant to one or more agents.

(v) Quality control. Susceptibility test quality control was achieved by including the appropriate reference strains and a control drug in each batch of tests; MICs for the reference strains were required to be within the acceptable limits of the National Committee for Clinical Laboratory Standards (16, 17) for the data from the test to be considered acceptable.

Time-kill assay. (i) Aerobic bacteria. The bacteriostatic or bactericidal effects of U-100592, U-100766, and the comparison drugs against 22 aerobic or facultative bacterial isolates were assessed by a time-kill assay. For enterococci and staphylococci, cultures were grown overnight at 35°C on MHA, and at approximately 15 min prior to the experiment, the colonies were harvested and a cell suspension equal to the turbidity of a 0.5 McFarland standard was prepared in deionized water. This suspension was diluted 1:9 in MHB to achieve a target cell concentration of approximately 10^7 CFU/ml. This suspension constituted the inoculum for the time-kill reaction tubes. For streptococci, an actively growing and more turbid cell suspension was prepared. Cultures were grown overnight on MHA supplemented with 5% defibrinated sheep blood, and at 90 min prior to the experiment, several colonies were transferred to 5 ml of prewarmed (35°C) supplemented proteose broth (SPB; proteose peptone no. 3 [Difco], 15 g/liter; dipotassium phosphate, 4 g/liter; potassium dihydrogen phosphate, 1 g/liter; sodium chloride, 5 g/liter; soluble starch [Difco], 1 g/liter; sodium bicarbonate, 420 mg/liter; IsoVitaleX [Becton Dickinson, Inc.], 10 ml/liter [pH 7.2]). Following the 90-min preincubation, the cultures were diluted with SPB to equal the turbidity of a 1.0 McFarland standard. The time-kill reaction tubes were prepared to contain 8.0 ml of the appropriate prewarmed broth medium (MHB for staphylococci and enterococci; SPB for streptococci), 1.0 ml of the antibacterial agent (diluted in the appropriate broth to a concentration 10-fold the final test concentration), and 1.0 ml of inoculum. The final drug concentration was fourfold the MIC for each test strain. An identical reaction tube that contained broth and inoculum, but no antibacterial agent, constituted the culture growth control. This procedure resulted in a cell concentration of about 10^6 to 10^7 CFU/ml for most cultures. The tubes were incubated at 35°C, and samples were removed for viable counts at 0, 6, and 24 h for all cultures except *Streptococcus pneumoniae*, which were sampled at 0, 3, and 6 h because of the autolytic nature of some strains during overnight incubation in broth. Viable counts for enterococci and staphylococci were determined by a semiautomated spiral plating method. A 0.5-ml sample was removed from the reaction tube at each time point, and serial dilutions (10^{-1}, 10^{-3}, and 10^{-5}) were prepared in MHB. The diluted samples were applied to the surfaces of duplicate agar plates by an Autoplate model 3000 spiral plater (Spiral Biotech, Inc., Bethesda, Md.). Following overnight incubation, the colonies were counted with a Laser Colony Scanner model 500A Bacterial Enumerator, and the viable count was calculated by using the associated CASBA II software (Spiral Biotech, Inc.). For the streptococci, a 0.5-ml sample was removed from the reaction tube at each time point and a series of 10-fold dilutions was prepared in SPB. A 100-μl volume of each dilution was applied to duplicate blood agar plates and was spread over the surface with a sterile glass hockey stick. Following overnight incubation at 35°C, the colonies were manually counted and the viable count was calculated. Results from sample dilutions yielding fewer than 20 colonies per plate were considered unreliable. Preliminary experiments demonstrated evidence of drug carryover on the viable count plates if the reaction tube samples were not diluted at least 1:9; therefore, throughout these experiments, undiluted samples were never used as the basis for a viable count. This resulted in a limit of detection of 2×10^3 CFU/ml for manual counts and 4.0×10^3 CFU/ml for the spiral dilution method, which was not as sensitive as might ideally be accomplished. However, with an initial inoculum size of 10^6 to 10^7 CFU/ml, it was still possible to assess a bactericidal effect as defined by a viable count reduction of greater than 3 log CFU/ml.

(ii) Anaerobic bacteria. The time-kill assay method used for anaerobic bacteria was similar to that used for aerobic bacteria, with the exception that most

manipulations and incubations took place in the Forma anaerobic glove box. Anaerobic Broth (AB; Difco) was prepared in bottles with vented caps and was allowed to prereduce in the chamber for at least 48 h prior to use. The anaerobic isolates *B. fragilis* ATCC 25285, *Clostridium perfringens* ATCC 13124, and *Peptostreptococcus magnus* 30980 were grown overnight on Wilkins-Chalgren agar plates (Difco) at 37°C in the glove box. The colonies were harvested, and a cell suspension equal to the turbidity of a 0.5 McFarland standard was prepared in AB. A 1:9 dilution in broth was prepared; this constituted the inocula for the experiments. The time-kill reaction tubes were prepared to contain 8.0 ml of AB, 1.0 ml of the drug solution (diluted in AB to a concentration 10-fold the final test concentration), and 1.0 ml of inoculum. The final drug concentration was fourfold the MIC for each test strain. An identical reaction tube that contained broth and inoculum, but no antibacterial agent, constituted the culture growth control. This procedure resulted in an initial cell concentration of about 10^6 to 10^7 CFU/ml. Viable counts were determined at 0, 6, and 24 h by a semiautomated spiral plating method. A 0.5-ml sample was removed from the reaction tube at each time point, and serial dilutions (10^{-1}, 10^{-3}, and 10^{-5}) were prepared. The diluted samples were applied onto the surfaces of duplicate Wilkens-Chalgren agar plates with the Autoplate model 3000 spiral plater. The plates were incubated in the Forma anaerobic chamber for 48 h at 37°C. Viable counts were determined by using the Laser Colony Scanner model 500A, as described above; the limit of detection was 4.0×10^3 CFU/ml.

Determination of spontaneous mutation frequency. *S. aureus* ATCC 29213 was swabbed onto the surfaces of two MHA plates and was grown for 20 h at 35°C. All of the growth from these two plates was harvested with sterile cotton swabs, and an extremely dense cell suspension was prepared in 5 ml of MHB. The viable count of this suspension was determined from an average for triplicate plates by the spiral dilution method described above. This cell suspension constituted the inoculum for the study. Serial dilutions of U-100592, U-100766, and rifampin were prepared in 1.0-ml volumes of sterile distilled water. To each 1.0-ml volume was added 9.0 ml of molten (47°C) MHA; the supplemented agar was mixed, poured into 15-by-100-mm petri dishes, and allowed to solidify and dry at room temperature prior to inoculation. Duplicate plates were prepared to contain the oxazolidinones at two-, four-, and eightfold the MICs; the control drug rifampin was prepared at fourfold the MIC. The plates were inoculated with 0.10 ml of the cell suspension, which was spread over the agar surface with a sterile glass hockey stick. Once the inoculum was absorbed into the agar, the plates were incubated at 35°C for 48 h. After incubation, the viable count of the inoculum and the number of colonies growing on the drug-supplemented plates at each concentration were determined and the spontaneous mutation frequency was calculated.

Resistance development. To determine whether repeated exposure of organisms to subinhibitory concentrations of U-100592 and U-100766 resulted in the rapid development of resistance, we used a spiral gradient, serial transfer method. Solutions of U-100592, U-100766, streptomycin, ciprofloxacin, and vancomycin were applied onto the surfaces of duplicate large petri dishes (150 by 25 mm) containing 50 ml of MHA with the Autoplate model 3000 spiral plater. The drug solutions were prepared at a concentration that yielded a spiral gradient in the agar plate of 35 to 0.08 μg/ml (except for streptomycin, which was prepared to yield a gradient of 350 to 0.80 μg/ml). The test cultures included *S. aureus* ATCC 29213, two strains of multiple-drug-resistant *S. aureus*, *E. faecalis* ATCC 29212, and one isolate of *Enterococcus faecium*. Cell suspensions were prepared in MHB to equal the turbidity of a 0.5 McFarland standard, and they were then incubated for 6 h at 35°C. Sterile swabs were used to make duplicate streaks, arranged like the spokes of a wheel, of each isolate onto the spiral gradient plates, and the plates were incubated for 42 to 66 h. Following incubation, the distance from the center of the plate to the point where heavy, confluent growth began was measured for each streak on each plate, and the four results for each antibacterial agent were averaged. Then, using a sterile swab, growth from the leading edge of the streak was harvested and used to inoculate a 5-ml tube of Trypticase soy broth (Difco) to equal the turbidity of a 0.5 McFarland standard. The cultures were grown for 6 h at 35°C, and the entire process was repeated on fresh drug plates; a total of six serial transfers were performed during a 2-week period.

RESULTS

In vitro susceptibility tests. The MICs of U-100592, U-100766, and the comparison drugs for 222 bacterial clinical isolates are summarized in Table 1. The oxazolidinones demonstrated strong activity against corynebacteria, enterococci, staphylococci, and streptococci. The potencies of U-100592 and U-100766 were generally comparable to (that is, the MICs at which 90% of isolates are inhibited [MIC_{90}s] were within one twofold dilution of vancomycin) or slightly less than that of vancomycin for all vancomycin-susceptible bacterial groups. Vancomycin-resistant enterococci of either the VanA or VanB resistance phenotypes were not cross-resistant to the oxazolidinones. The oxazolidinones and vancomycin demonstrated potent activities against methicillin-resistant staphylococci and penicillin-resistant pneumococci. Vancomycin demonstrated poor activity against the fastidious gram-negative species *H. influenzae* and *M. catarrhalis*, while the oxazolidinones demonstrated strong activity against *M. catarrhalis* (MIC_{90}, 4 μg/ml) and modest activity (MICs, 4 to 16 μg/ml) against *H. influenzae*. Both oxazolidinones were active against *B. fragilis*, with U-100766 being the more active of the two agents. Excluding *B. fragilis*, U-100592 appeared to be slightly more active overall than U-100766. A strain-by-strain comparison (data not shown) showed that the two agents had identical MICs for 46% of the test strains; U-100766 was twofold less active than U-100592 against 44% of the test strains and was twofold more active against 7% of the test strains. For the remaining strains, U-100766 was fourfold more active than U-100592 against two strains (0.9%) and fourfold less active against three strains (1.4%).

The MICs of U-100592, U-100766, and vancomycin for a number of individual isolates of aerobic bacteria that were not included in Table 1 are summarized in Table 2. The oxazolidinones demonstrated strong activity against *Bacillus* spp., staphylococci, and enterococci (including strains of the VanC resistance phenotype). The oxazolidinones also inhibited *Staphylococcus haemolyticus* UC 12198, a vancomycin-intermediate isolate, at 1 μg/ml. Neither the oxazolidinones nor vancomycin was active against representative strains of the gram-negative species *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus penneri*, and *Pseudomonas aeruginosa* (data not shown).

The activities of the oxazolidinones, vancomycin, and clindamycin against individual isolates of anaerobic bacteria are summarized in Table 3. Clindamycin demonstrated potent broad-spectrum antianaerobe activity, as expected. Vancomycin was virtually inactive against the gram-negative anaerobes but had potent activity against the gram-positive anaerobes. U-100592 and U-100766 demonstrated activity similar to that of vancomycin against the gram-positive anaerobes.

The oxazolidinones were very active against *M. tuberculosis*, as shown in Table 4. These agents were potent inhibitors of both drug-susceptible and drug-resistant isolates and did not appear to be cross-resistant with standard antitubercular agents.

Effect of serum on MIC. The effect of pooled human serum on the broth microdilution MICs of the oxazolidinones was compared with those of novobiocin, clindamycin, and vancomycin. Novobiocin was included as an example of a highly protein-bound antibiotic (95 to 99%) whose activity is antagonized in vitro by the presence of serum. Clindamycin is also a highly protein-bound agent, but to a lesser degree than novobiocin (87 to 95%), while vancomycin is considered to have a moderate degree of protein binding (approximately 55%) (8). U-100592 and U-100766 are bound in human plasma at approximately 6 and 31%, respectively (6, 7). The data demonstrated the antagonism of novobiocin activity by pooled human serum, as expected; MICs for all organisms increased significantly (greater than fourfold) with increasing percentages of serum. For clindamycin, a relatively small but significant increase was evident for one test strain, *S. aureus* ATCC 29213, although the MIC still fell within the range for susceptibility to clindamycin. Vancomycin MICs were generally not affected by the presence of serum, although in one instance (*E. faecalis* 30536) there was a significant increase in the MIC (from 0.50 to 2 μg/ml), which occurred in the presence of 40% serum. U-100592 and U-100766 activities were not significantly influenced by the presence of serum for any of the cultures tested.

TABLE 1. In vitro activities of U-100592, U-100766, and comparison drugs

Organism (no. of strains)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
Corynebacterium spp. (11)	U-100592	0.25–0.5	0.25	0.5
	U-100766	0.5–1	0.5	0.5
	Vancomycin	0.5–1	1	1
Enterococcus faecalis, vancomycin susceptible (14)	U-100592	2–4	2	2
	U-100766	2–4	4	4
	Vancomycin	0.5–2	1	2
E. faecalis VanB (10)	U-100592	1–4	2	2
	U-100766	2–4	2	4
	Vancomycin	16–>16	>16	>16
Enterococcus faecium, vancomycin susceptible (9)	U-100592	1–2	2	
	U-100766	2–4	4	
	Vancomycin	0.25–2	0.5	
E. faecium VanA (16)	U-100592	0.5–4	2	2
	U-100766	1–4	2	4
	Vancomycin	>16	>16	>16
E. faecium VanB (14)	U-100592	1–4	2	2
	U-100766	2–4	2	4
	Vancomycin	>16	>16	>16
Haemophilus influenzae (9)	U-100592	4–16	8	
	U-100766	8–16	8	
	Vancomycin	>16	>16	
	Ampicillin	0.5–>16	0.5	
Listeria monocytogenes (10)	U-100592	4–8	4	8
	U-100766	2–4	2	2
	Vancomycin	1–2	1	1
Moraxella catarrhalis (10)	U-100592	2–4	4	4
	U-100766	4–8	4	4
	Vancomycin	>16	>16	>16
	Ampicillin	≤0.25–4	0.125	1
Staphylococcus aureus, methicillin susceptible (12)	U-100592	2–8	4	4
	U-100766	4	4	4
	Vancomycin	0.5–1	1	1
S. aureus, methicillin resistant (41)	U-100592	2–8	4	4
	U-100766	2–16	4	4
	Vancomycin	0.5–2	1	2
Staphylococcus epidermidis, methicillin susceptible (11)	U-100592	1–2	1	2
	U-100766	1–2	2	2
	Vancomycin	1–2	2	2
Staphylococcus epidermidis, methicillin resistant (13)	U-100592	1	1	1
	U-100766	1–2	2	2
	Vancomycin	1–4	2	2
Streptococcus pneumoniae, penicillin susceptible (10)	U-100592	≤0.25–1	0.5	0.5
	U-100766	0.5–1	0.5	1
	Vancomycin	≤0.25–0.5	≤0.25	≤0.25
Streptococcus pneumoniae, penicillin intermediate and resistant (10)	U-100592	≤0.25–0.5	0.5	0.5
	U-100766	0.5–1	1	1
	Vancomycin	≤0.25–0.5	≤0.25	≤0.25
Streptococcus pyogenes (12)	U-100592	0.5–1	1	1
	U-100766	1–2	1	2
	Vancomycin	≤0.25–0.5	≤0.25	0.5
Bacteroides fragilis (10)	U-100592	8–>16	16	16
	U-100766	4–8	4	4
	Vancomycin	>16	>16	>16
	Clindamycin	0.25–>16	≤0.25	1

TABLE 2. Comparative in vitro activities of U-100592, U-100766, and vancomycin

Organism	Culture no.	MIC (μg/ml)		
		U-100592	U-100766	Vancomycin
Bacillus cereus	UC 3145	1	1	2
Bacillus subtilis	UC 564	1	1	≤0.25
Enterococcus casseliflavus VanC	31266	1	2	8
Enterococcus gallinarum VanC	31294	2	4	8
Staphylococcus capitis	31527	0.50	≤0.50	1
Staphylococcus haemolyticus	UC 12197	1	1	4
Staphylococcus haemolyticus	UC 12198[a]	1	1	8
Staphylococcus haemolyticus	31261	0.50	1	1
Staphylococcus intermedius	UC 12596	2	2	1
Staphylococcus saprophyticus	31115	4	4	1
Staphylococcus simulans	UC 12672	1	1	2
Staphylococcus warneri	31258	1	2	1

[a] Noninducible, low-level resistance to vancomycin.

Time-kill assays. The results of the time-kill kinetic studies are summarized in Table 5. The data are presented in terms of the log-CFU-per-milliliter change (positive or negative) and are judged relative to the conventional definition of bactericidal activity, that is, a 3.0-log-CFU/ml or greater reduction in the initial inoculum by 24 h. For enterococci, the oxazolidinones demonstrated bacteriostatic activity, as did vancomycin. The lack of in vitro bactericidal activity for vancomycin against vancomycin-susceptible enterococci has been described previously (14, 21). The oxazolidinones were inhibitors of both vancomycin-susceptible and vancomycin-resistant strains of enterococci. The oxazolidinones also demonstrated bacteriostatic activity against the staphylococci (with the exception of a bactericidal result for U-100592 and *Staphylococcus simulans* UC 12672); however, the degree of killing was greater than that seen for the enterococci, with most strains demonstrating a 1.0-log-CFU/ml reduction or greater. Vancomycin achieved at least a 3-log-CFU/ml reduction for 5 of 10 isolates and a 2- to 3-log-CFU/ml reduction for the rest of the strains. Against streptococci, U-100592 was bactericidal for all five test strains, while U-100766 was bactericidal for three strains and reduced the counts of the remaining strains from 2.5 to 2.9 log CFU/ml. Vancomycin was bactericidal, or very nearly bactericidal (i.e., a reduction of 2.9 log CFU/ml), for four of five test strains. Penicillin G was bactericidal for all strains except the penicillin-resistant isolate. The oxazolidinones were bactericidal for *B. fragilis* and *C. perfringens*, as was clindamycin. Vancomycin produced a bactericidal effect for *C. perfringens*, but it was not tested against *B. fragilis* because of its lack of inherent activity. All of the test agents produced a similar degree of killing (reduction of 2.0 to 2.4 log CFU/ml) for *P. magnus.*

TABLE 4. Activities of U-100592 and U-100766 against *M. tuberculosis*

Compound	Organism group[a]	No. of strains inhibited at concn (μg/ml) of:		
		0.125	0.50	2
U-100592	S	1	4	
	R		4	1
U-100766	S		5	
	R		3	2

[a] S, group of five drug-susceptible isolates (including susceptibility to streptomycin, isoniazid, rifampin, ethionamide, and ethambutol); R, group of five multiple-drug-resistant isolates (including resistance to one or more of the agents listed above).

Spontaneous mutation frequency and resistance development. No colonies resistant to U-100592 or U-100766 at two-, four-, or eightfold the MIC were detected, yielding spontaneous mutation frequencies for *S. aureus* ATCC 29213 of $<3.8 \times 10^{-10}$ and $<8 \times 10^{-11}$, respectively. The test control, rifampin, yielded a spontaneous mutation frequency of 4.3×10^{-8} at fourfold the MIC. The spiral gradient, serial passage experiments (data not shown) clearly produced a rapid development of resistance of staphylococci to the control agents streptomycin and ciprofloxacin. With the exception of one isolate, there was no evidence of spontaneous mutation or a rapid development of resistance to either the oxazolidinones or vancomycin for any of the cultures tested by the spiral gradient method. One strain of *E. faecalis* grew to the center of the U-100766 spiral gradient plate, but only after five serial passages.

DISCUSSION

Bacterial resistance is a significant nosocomial problem and is of increasing importance in community-acquired infections. The greatest threat to successful antibiotic treatment and the impetus behind the search for new antibiotics and other treatment modalities are the evolution and spread of antibiotic resistance. Oxazolidinones are a novel class of investigational antibacterial agents, and by virtue of their unique mechanisms of action, they are not cross-resistant with other classes of antibacterial agents for organisms within their spectra of activity. Thus, a clinically useful oxazolidinone could represent

TABLE 3. Comparative in vitro activities of U-100592, U-100766, vancomycin, and clindamycin against anaerobic bacteria

Organism	Culture no.	MIC (μg/ml)			
		U-100592	U-100766	Vancomycin	Clindamycin
Bacteroides distasonis	UC 6518	4	4	>16	0.125
Bacteroides fragilis	ATCC 25285	8	4	>16	0.5
Bacteroides thetaiotaomicron	30557	16	4	>16	4
Bacteroides vulgatus	UC 9454	8	2	16	0.06
Fusobacterium nucleatum	UC 6324	0.5	0.5	>16	0.125
Prevotella bivia	31005	1	1	>16	<0.015
Prevotella melaninogenica	UC 6523	1	1	>16	<0.015
Peptostreptococcus asaccharolyticus	UC 6319	0.5	0.5	0.25	0.03
Peptostreptococcus magnus	UC 9453	2	2	0.5	1
Clostridium perfringens	ATCC 13124	2	2	1	0.06
Clostridium difficile	30315	2	2	2	8

TABLE 5. Time-kill results for oxazolidinones and comparison drugs tested at fourfold the MIC[a]

Organism[b]	Log decrease (increase) at endpoint for the following antibiotic[c]:					
	U-100592	U-100766	Vancomycin	Penicillin G	Clindamycin	Control
Enterococcus faecalis ATCC 29212	0.3	0.1	1.6			(1.1)
30536	0.7	0.3	0.1			(1.2)
31621 (VanA)	0.3	0.2	(1.4)			(1.2)
31227 (VanB)	0.9	0.4	(1.3)			(1.4)
Enterococcus faecium UC 12712	0.6	0.5	0.3			(1.5)
31274 (VanA)	0.9	0.2	(1.8)			(1.7)
31275 (VanA)	0.6	0.6	(1.0)			(1.4)
Staphylococcus aureus ATCC 29213	1.2	1.1	3.0			(1.6)
UC 9271	0.5	0.6	>3.5			(1.3)
UC 12673 (MRSA)	0.9	0.9	2.8			(1.7)
UC 6685 (MRSA)	1.7	1.7	2.9			(1.7)
Staphylococcus epidermidis 30031	1.4	1.2	2.3			(1.9)
30671	0.6	1.2	2.5			(1.9)
UC 12084 (MRSE)	1.5	2.5	>3.6			(1.1)
31292 (MRSE)	1.4	0.9	>3.2			(1.7)
Staphylococcus haemolyticus UC 12198	2.3	2.1	2.1			(1.8)
Staphylococcus simulans UC 12672	3.0	2.6	>3.3			(1.1)
Streptococcus pneumoniae ATCC 6305 (Pen^s)	3.2	3.3	2.9	>4.2		(1.4)
31229 (Pen^i)	>4.1	>4.1	>4.2	>4.2		(0.7)
31135 (Pen^r)	3.3	2.5	2.0	(0.8)		(1.8)
Streptococcus pyogenes UC 152	3.5	3.4	2.9	>4.1		(1.3)
Streptococcus agalactiae 30048	3.4	2.9	4.3	3.8		(1.7)
Bacteroides fragilis ATCC 25285	>3.5	>3.5			>3.5	(1.1)
Clostridium perfringens ATCC 13124	>3.7	>3.6	>3.7		>3.7	(2.3)
Peptostreptococcus magnus 30980	2.0	2.0	2.4		2.0	(1.2)

[a] Aerobic bacteria were tested at fourfold MIC or the following maximum concentrations: penicillin, 2.0 μg/ml; vancomycin, 4.0 μg/ml; oxazolidinone, 8.0 μg/ml.
[b] Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; Pen, penicillin; s, susceptible; i, intermediate; r, resistant.
[c] The log-CFU-per-milliliter reduction was calculated at 24 h for all cultures except *Streptococcus pneumoniae*, which was calculated at 6 h. Parentheses indicate an increase in the viable count.

an advance in antimicrobial therapy, particularly for problematic resistant organisms that are becoming widespread. U-100592 and U-100766, novel piperazinyl and morpholinyl oxazolidinones, have demonstrated in vitro inhibitory activities against a variety of clinically important bacterial species. Both analogs have a low degree of serum protein binding, and their inhibitory activities are not diminished in vitro by pooled human serum. Thus, it appears likely that the possibility for undesirable interactions with specific serum components is limited. While susceptibility breakpoints cannot be defined until the completion of human clinical trials, the in vitro susceptibility data presented herein indicate that the useful spectra of activity of U-100592 and U-100766 might include staphylococci (including methicillin-resistant strains), enterococci (including vancomycin-resistant strains of all known phenotypes), streptococci (including penicillin-resistant pneumococci), corynebacteria, *M. tuberculosis* (including multiply-resistant strains), and some species of anaerobic bacteria. The fastidious gram-negative aerobe *M. catarrhalis* might also be considered susceptible.

Conventional time-kill data demonstrated the species-specific bacteriostatic or bactericidal activities of these agents. While bactericidal activity might be intuitively preferable, both primarily bacteriostatic antibiotics (such as lincosamides, macrolides, tetracyclines, and chloramphenicol) and bactericidal antibiotics (such as β-lactams, aminoglycosides, quinolones, and vancomycin) have proven clinical utility for a wide variety of indications. The in vitro data have correlated well with the reported efficacies of the compounds in experimental models of bacteremia and soft tissue infections (13); however, since U-100592 and U-100766 are representatives of a new class of antibacterial agents, studies are also needed to determine which pharmacodynamic relationship (e.g., maximum concentration of drug in serum:MIC, area under the concentration-time curve:MIC, and time or percentage of dosing interval above MIC) is critical for in vivo efficacy.

Further developmental studies with these new agents are warranted. Phase I human trials are in progress, and a phase II program is being planned.

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